fold the weight of water. Dilute 1500 μL brain homogenate with 500 μL 1 M pH 9 phosphate buffer, add 8 mL diethyl ether, extract. Evaporate the organic layer, dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 6 Intersil ODS-2
Mobile phase: MeCN:0.018% TFA 20:80 (plasma), MeCN:0.018% TFA 15:85 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 300

CHROMATOGRAM
Limit of quantitation: 5 ng/mL (plasma), 40 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE
Matrix: solutions

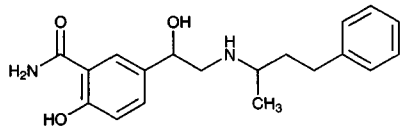
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 14.72

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Labetalol



Molecular formula: $C_{19}H_{24}N_2O_3$
Molecular weight: 328.41
CAS Registry No.: 36894-69-6, 32780-64-6 (HCl)
Merck Index: 5341
Lednicer No.: 3 24; 4 20

SAMPLE
Matrix: amniotic fluid, blood, fetal tracheal fluid
Sample preparation: 50-500 μL Plasma, fetal tracheal fluid, or amniotic fluid + water to 750 μL total volume, add 500 μL pH 9.5 carbonate buffer, add 6 mL ethyl acetate, extract. Remove the organic layer and extract it with 600 μL 10 mM phosphoric acid, inject a 60 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 10 × 3 Chiral AGP (Regis)
Column: 100 × 4 10 μm Chiral AGP (Regis)
Mobile phase: 20 mM phosphate buffer containing 15 mM tetrabutylammonium phosphate, degas with helium for 30 min, adjust pH to 7.10 with phosphoric acid
Flow rate: 0.5
Injection volume: 60
Detector: F ex 230 em 400

CHROMATOGRAM
Retention time: 19 (SR), 23 (SS), 28 (RS), 34 (RR)
Limit of detection: 0.15 ng

KEY WORDS
plasma; sheep; diastereomers; chiral

REFERENCE
Doroudian,A.; Yeleswaram,K.; Rurak,D.W.; Abbott,F.S.; Axelson,J.E. Sensitive high-performance liquid chromatographic method for direct separation of labetalol stereoisomers in biological fluids using an α_1-acid glycoprotein stationary phase, *J.Chromatogr.*, **1993**, *619*, 79–86.

SAMPLE
Matrix: bile, perfusate
Sample preparation: 500 μL Perfusate or 100 μL bile + 1 mL 1 M pH 10.3 carbonate buffer + 5 mL acid-washed diethyl ether, vortex, centrifuge. Remove the organic layer and add it to 125 μL 0.5% phosphoric acid, extract, inject a 10 μL aliquot of the aqueous layer. (Deconjugate 500 μL perfusate with 250 μL 8000 U/mL β-D-glucuronidase/aryl sulfatase in 200 mM pH 4.5 sodium acetate buffer, heat at 40° for 1 h, proceed as above.)

HPLC VARIABLES
Column: 100 × 8 4 μm Novapak phenyl radial compression
Mobile phase: MeCN:water:triethylamine 23:77:1 adjusted to pH 3.6 with concentrated phosphoric acid
Flow rate: 3
Injection volume: 10
Detector: F ex 295 em 360

CHROMATOGRAM
Retention time: 6.9
Internal standard: labetalol

OTHER SUBSTANCES
Extracted: propranolol

KEY WORDS
sheep; liver; labetalol is IS

REFERENCE
Ring,J.A.; Ghabrial,H.; Ching,M.S.; Shulkes,A.; Smallwood,R.A.; Morgan,D.J. Fetal hepatic propranolol metabolism. Studies in the isolated perfused fetal sheep liver, *Drug Metab.Dispos.*, **1995**, *23*, 190–196.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL water + 100 μL 20% sodium metabisulfite (freshly prepared) + 1 mL 1 M pH 10.2 sodium carbonate + 8 mL ether, shake gently for 10 min on a reciprocating shaker, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, centrifuge for 4 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 10 mM potassium phosphate adjusted to pH 3.4 with 5 M HCl.)
Injection volume: 50
Detector: F ex 310 em 380 (filter)

CHROMATOGRAM
Retention time: 4.8
Internal standard: labetalol

OTHER SUBSTANCES
Extracted: propranolol

KEY WORDS
plasma; labetalol is IS

REFERENCE
Drummer,O.H.; McNeil,J.; Pritchard,E.; Louis,W.J. Combined high-performance liquid chromatographic procedure for measuring 4-hydroxypropranolol and propranolol in plasma: Pharmacokinetic measurements following conventional and slow-release propranolol administration, *J.Pharm.Sci.*, **1981**, *70*, 1030–1032.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL buffer + 10 mL chloroform:isopropanol 100:2 , shake on a horizontal reciprocal shaker at 30 strokes/min for 10 min, centrifuge at 1000 g for 10 min. Remove 8 mL of the organic phase and evaporate it to dryness under a stream of nitrogen, dissolve the residue in 50 μL MeCN:MeOH:100 mM HCl 30:20:5, inject a 25 μL aliquot. (Buffer was 3.80 g/L sodium bicarbonate and 0.504 g/L sodium carbonate, pH 8.8.)

HPLC VARIABLES
Column: 150 × 6 5 μm Asahipack ODP 50 (octadecyl-bonded polymer gel)
Mobile phase: MeCN:50 mM pH 11.5 diethylamine 84:16 which was 36 mM in NaCl
Flow rate: 0.9
Injection volume: 25
Detector: F ex 340 em 389

CHROMATOGRAM
Retention time: 9.5 (RR-SS), 10.5 (RS-SR)
Limit of detection: 3 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics; diastereomers

REFERENCE
Grellet,J.; Michel-Gueroult,P.; Ducint,D.; Saux,M.C. Sensitive high-performance liquid chromatographic method for the determination of labetalol diastereoisomers in plasma samples without derivatization, *J.Chromatogr.B*, **1994**, *652*, 59–66.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of

the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM

Retention time: 4.58
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: bulk
Sample preparation: 1 mg Labetalol + 3 mg reagent + 100 μL MeCN + 50 μL water + 3 μL triethylamine, vortex, heat at 60° for 1 h. Remove a 150 μL aliquot and add it to 400 μL MeCN, vortex, inject a 5 μL aliquot. (Prepare the reagent, (4S-cis)-2,2-dimethyl-5-isothiocyanato-4-

phenyl-1,3-dioxane (PHEDIT), as follows. Add dropwise 5 g (4S,5S)-(+)-5-amino-2,2-dimethyl-4-phenyl-1,3-dioxane in 150 mL dichloromethane to 5 g 1,1'-thiocarbonyldiimidazole stirred in 150 mL dichloromethane, stir at room temperature for 3 h, wash the reaction mixture three times with 300 mL portions of 5% sodium bicarbonate, wash three times with 300 mL portions of water. Dry the organic layer over anhydrous sodium sulfate for 20 min, evaporate to dryness under reduced pressure, recrystallize from EtOH to give (4S-cis)-2,2-dimethyl-5-isothiocyanato-4-phenyl-1,3-dioxane as a pale yellow solid (mp 105-6°).)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:20 mM pH 4.60 $(NH_4)H_2PO_4$ 63:37
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 19.7 (RS or SR), 21.4 (RS or SR), 23.8 (SS), 30.0 (RR)

KEY WORDS
derivatization; chiral; comparison with other derivatization reagents

REFERENCE
Desai,D.M.; Gal,J. Reversed-phase high-performance liquid chromatographic separation of the stereoisomers of labetalol via derivatization with chiral and non-chiral isothiocyanate reagents, *J.Chromatogr.*, **1992**, *579*, 165–171.

SAMPLE
Matrix: bulk
Sample preparation: 1 mg Labetalol + 3 mg 1-naphthalenemethyl isothiocyanate (Trans World Chemicals, Chevy Chase MD) + 100 μL MeCN + 50 μL water + 3 μL triethylamine, vortex, heat at 60° for 1 h. Remove a 150 μL aliquot and add it to 400 μL MeCN, vortex, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:20 mM pH 4.60 $(NH_4)H_2PO_4$ 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 10.42 (RS/SR), 14.73 (SS/RR)

KEY WORDS
derivatization; comparison with other derivatization reagents

REFERENCE
Desai,D.M.; Gal,J. Reversed-phase high-performance liquid chromatographic separation of the stereoisomers of labetalol via derivatization with chiral and non-chiral isothiocyanate reagents, *J.Chromatogr.*, **1992**, *579*, 165–171.

SAMPLE
Matrix: formulations, urine
Sample preparation: Urine. Condition a Bond Elut Certify LRC SPE cartridge with 6 mL water. Mix 3.75 mL urine with 750 μL 1 M pH 9.0 borate buffer, centrifuge at 734 g for 5 min. Add a 3 mL aliquot to the SPE cartridge. Wash with 2 mL water, 1 mL 100 mM pH 4.0 acetate buffer, and 2 mL MeOH using vacuum (5 mmHg). Dry the SPE cartridge under vacuum (<150 mmHg) for 5 min. Elute with 2 mL 2% ammonia in chloroform:isopropanol 60:40 using vacuum (2 mmHg) (Caution! Chloroform is a carcinogen!). Evaporate the eluate to dryness under a gentle stream of nitrogen at 60°. Dissolve the residue in 1 mL mobile phase, inject an aliquot. Tablets. Crush and mix tablets to a fine powder, weigh, dissolve in water, shake for 20 min,

filter (Whatman No. 41 filter-paper), wash, make up to a fixed volume. Dilute an aliquot with mobile phase and inject an aliquot.

HPLC VARIABLES
Guard column: μ-Bondapak C18
Column: 250 × 4.6 5 μm Supelcosil ABZ + Plus
Mobile phase: MeCN:water 30:70 containing 5 mM acetate buffer adjusted to pH 4.5 with acetic acid or 1 M KOH
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: E, PAR Model 400, glassy carbon cell + 1.3 V, Ag/AgCl reference electrode, platinum auxiliary electrode in the DC mode with 5-s low-pass filter time constant, current range 20-100 nA

CHROMATOGRAM
Retention time: 5.95
Limit of detection: 5 ng/mL (solutions), 10 ng/mL (urine)
Limit of quantitation: 20 μg/mL (urine)

KEY WORDS
tablets; SPE

REFERENCE
Ceniceros,C.; Maguregui,M.I.; Jiménez,R.M.; Alonso,R.M. Quantitative determination of the β-blocker labetalol in pharmaceuticals and human urine by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1998**, *705*, 97–103.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 40:60 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 3.33 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.67

OTHER SUBSTANCES
Also analyzed: bisoprolol, carvedilol, metipranolol, oxprenolol, talinolol, toliprolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:40 mM pH 4.3 acetate buffer 75:25
Flow rate: 1.5
Injection volume: 20
Detector: UV 278

OTHER SUBSTANCES
Extracted: dextromethorphan

REFERENCE
Abdel-Moety,E.M.; Al-Deeb,O.A.; Khattab,N.A. Determination of dextromethorphan hydrobromide in bulk form and dosage formulations by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 4127–4134.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 60:35:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 308

CHROMATOGRAM
Retention time: 23, 25, 28, 33 (diastereomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.68 (A), 4.22 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, meth-

yldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: tissue
Sample preparation: Weigh out brain tissue and homogenize in 4 volumes 400 mM perchloric acid using a Tamson motor-driven PTFE/glass homogenizer at 1400 rpm to give a final tissue concentration of 25 mg/mL in the perchloric acid. For each 1 mL of homogenate add 40 μL 1.48 μg/mL propranolol in 200 mM sulfuric acid, centrifuge at 3000 g for 15 min. Remove 1 mL supernatant and add it to 10 μL 10 M NaOH and 350 μL buffer, vortex for 10 s, add 8 mL diethyl ether, shake mechanically for 45 min, centrifuge at 2000 g for 8 min. Remove the organic layer and add it to 200 μL 200 mM sulfuric acid, shake mechanically for 15 min, centrifuge at 2000 g for 8 min. Remove the aqueous layer and heat it at 45° for 1 h to remove traces of ether, inject a 50 μL aliquot. (Buffer was 90 g sodium carbonate and 32 g potassium carbonate in 1 L, pH 9.0.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm LC-18-DB (Supelchem)
Column: 250 × 4.6 5 μm LC-18-DB (Supelchem)
Mobile phase: MeCN:50 mM NaH_2PO_4:triethylamine 35:65:0.1, adjusted to pH 3.0 with orthophosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 5.5
Internal standard: propranolol (7.3)
Limit of detection: 33 ng/mL

OTHER SUBSTANCES
Simultaneous: clenbuterol

KEY WORDS
rat; brain

REFERENCE
Botterblom,M.H.A.; Feenstra,M.G.P.; Erdtsieck-Ernste,E.B.H.W. Determination of propranolol, labetalol and clenbuterol in rat brain by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 121–126.

SAMPLE
Matrix: urine

Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES

Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM

Retention time: 14.2
Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol, timolol

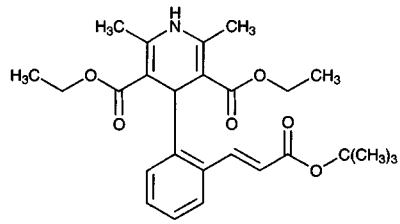
KEY WORDS

column-switching

REFERENCE

Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

Lacidipine



Molecular formula: $C_{26}H_{33}NO_6$
Molecular weight: 455.55
CAS Registry No.: 103890-78-4
Merck Index: 5344
Lednicer No.: 5 82

SAMPLE

Matrix: blood
Sample preparation: Condition a 500 mg Bondelut C18 SPE cartridge with 3 mL MeOH and 2 mL MeCN:water 50:50. 3 mL Plasma + 3 mL MeCN, centrifuge, add the supernatant to the SPE cartridge, wash with 2 mL MeCN:water 50:50, wash with 1.5 mL basic washing solution, wash with 1.5 mL acid washing solution, wash with 2 mL MeCN:water 50:50, elute with two 1.5 mL portions of MeCN. Evaporate the eluate, reconstitute in 100 μL mobile phase, store at 4°, inject an 82 μL aliquot. (Basic washing solution was MeCN:water:33% ammonia 10:88:2. Acid washing solution was MeCN:water:88% orthophosphoric acid 10:89:1.)

HPLC VARIABLES

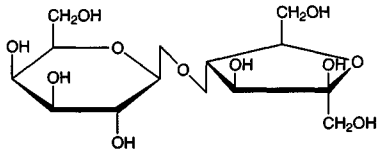
Guard column: 30 × 4.6 30-40 μm RP-8 (Merck)
Column: 60 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 6:66:28
Column temperature: 40
Flow rate: 1
Injection volume: 82
Detector: UV 300 or RIA

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.5 ng/mL

KEY WORDS
plasma; human; dog; rat; horse; protect from light; pharmacokinetics; SPE; automation of this procedure reported (see J. Chromatogr. B 1995; 669; 383)

REFERENCE
Pellegatti,M.; Braggio,S.; Sartori,S.; Franceschetti,F.; Bolelli,G.F. Validation of a high-performance liquid chromatographic-radioimmunoassay method for the determination of lacidipine in plasma, *J.Chromatogr.*, **1992**, *573*, 105–111.

Lactulose



Molecular formula: $C_{12}H_{22}O_{11}$
Molecular weight: 342.30
CAS Registry No.: 4618-18-2
Merck Index: 5360

SAMPLE
Matrix: beverages, juice, milk
Sample preparation: Orange juice. Dilute orange juice 100-fold with water, filter (Millipore HV, 0.45 μm), dilute filtrate 10-fold, inject an aliquot. Beverages. Dilute soft drinks 1000-fold with water, inject an aliquot. Milk. Dilute 5 mL milk to 100 mL with mobile phase, filter (Millipore HV, 0.45 μm), dilute filtrate 50-fold, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Cation H (Bio-Rad)
Column: 300 × 3.8 9 μm HPX 87-H Aminex (Bio-Rad)
Mobile phase: 10 mM Sulfuric acid
Column temperature: 50
Flow rate: 0.5
Injection volume: 40
Detector: E following post-column reaction, Hewlett-Packard 1049A programmable electrochemical detector, Metrohm detector cell, cuprous oxide working electrode +550 mV, glassy carbon auxiliary electrode, Ag/AgCl (3 M KCl) reference electrode. The column effluent mixed with 200 mM NaOH pumped at 0.4 mL/min, the mixture flowed through a 220 × 0.8 single-bead string reactor packed with 0.6 mm glass beads to the detector. (Prepare cuprous oxide electrode as follows. Stir 300 mg conductive carbon cement (Gerhard Neubauer, Münster), 60 mg cuprous oxide (Fluka), and 300 μL acetone until a thick paste forms as the acetone evaporates. Pack conductive carbon cement into the base of a 3 mm diameter cavity carbon paste electrode base (Metrohm), allow to dry, polish with dry emery paper (grade 2/0, Oakey), remove surface layer with an acetone-soaked tissue, pack the paste into the cavity, allow to dry overnight, polish with dry emery paper (grade 2/0), 3 μm imperial micro finishing film sheet (3M), 0.3 μm imperial micro finishing film sheet (3M), and 0.05 μm alumina particles on a Buehler pad, sonicate for 2 min in water (Anal. Chim. Acta 1995, 300, 5).)

CHROMATOGRAM
Retention time: 9.50
Limit of detection: 2 μM

OTHER SUBSTANCES
Also analyzed: arabinose, cellobiose, dextrose, fructose, fucose, galactitol, galactose, galacturonic acid, lactose, lyxose, maltose, mannitol, mannose, myo-inositol, raffinose, rhamnose, ribose, sorbose, sucrose, xylose

KEY WORDS
orange juice; soft drinks; post-column reaction; fruit

REFERENCE
Huang,X.; Pot,J.J.; Kok,W.T. Determination of sugars by liquid chromatography and amperometric detection with a cuprous oxide modified electrode, *Chromatographia*, **1995**, *40*, 684–689.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Dilute urine 1:10 to 1:40 with water, add a 1 mL aliquot to 1 mL 250 μg/mL melibiose, add Amberlite IR-120 H^+ to occupy one third of the volume, inject a 25 μL aliquot of the supernatant. Plasma. 200 μL Plasma + 200 μL 250 μg/mL melibiose, mix, add 200 μL ice cold 35 mg/mL 5-sulfosalicylic acid, let stand on ice for 20 min, centrifuge at 9000 g for 5 min, mix with Amberlite IR-120 H^+:Amberlite IRA 400 Cl^- 40:60, centrifuge, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Carbopac PA-1 (Dionex)
Column: 250 × 40 Carbopac PA-1 (Dionex)
Mobile phase: 120 mM NaOH containing 0.5 mM zinc acetate (urine) or 160 mM NaOH containing 0.675 mM zinc acetate (plasma) (At the end of each plasma sample wash with 1 M NaOH for 4 min.)
Flow rate: 1
Injection volume: 25
Detector: E, Dionex pulsed electrochemical detector, detection potential -0.01 V (0-0.5 s), oxidation potential +0.75 V (0.51-0.64 s), reduction potential -0.75 V (0.65-0.75 s), integration period 0.05-0.5 s

CHROMATOGRAM
Retention time: 5.9 (plasma), 6.9 (urine)
Internal standard: melibiose (4.0 (plasma), 4.6 (urine))
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: mannitol, 3-O-methylglucose, dextrose

KEY WORDS
plasma

REFERENCE
Fleming,S.C.; Kynaston,J.A.; Laker,M.F.; Pearson,A.D.J.; Kapembwa,M.S.; Griffin,G.E. Analysis of multiple sugar probes in urine and plasma by high-performance anion-exchange chromatography with pulsed electrochemical detection. Application in the assessment of intestinal permeability in human immunodeficiency virus infection, *J.Chromatogr.*, **1993**, *640*, 293–297.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Shodex Sugar SP 0810P and SP 0810
Mobile phase: water
Column temperature: 80
Flow rate: 0.5
Detector: RI

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fructose, galactose, glycerol, lactose, mannitol, pullulan P-10, raffinose, sorbitol, stachyose, sucrose, xylitol

REFERENCE

Majors,R.E. Polymeric liquid chromatography column technology in Japan, *LC.GC*, **1993**, *11*, 778–788.

SAMPLE

Matrix: urine

Sample preparation: Condition a 600 mg Maxi-Clean C18 SPE cartridge with 5 mL MeOH and 5 mL water. Add 2-3 mL urine to the SPE cartridge and pass it through the cartridge. Discard the first 1 mL, collect the residual volume and dilute it 1:1 with water. Dilute a 200 μL aliquot with 1.8 mL water containing 75 μm/mL cellobiose, add 400 g/L Amberlite IRA-400 resin Cl^- form (Fluka). Vortex for 10 s, centrifuge at 3000 g for 2 min. Filter (Micro-spin centrifuge cartridge, Nylon 66, 0.2 μm, Alltech) a 400 μL aliquot of the supernatant while centrifuging at 3000 g for 5 min. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Benson Carbohydrate BC-100 Ca^{2+} (Alltech)
Column: 300 × 6.5 10 μm Alltech 700 CH Carbohydrate (Alltech)
Mobile phase: Water
Column temperature: 85
Flow rate: 0.5
Injection volume: 50
Detector: ELSD, Varex MKIII (Alltech), drift tube temperature 120°, carrier gas flow (air) 41.67 cm^3/s

CHROMATOGRAM

Retention time: 8.88
Internal standard: cellobiose (7.55)
Limit of detection: 820 ng/mL

OTHER SUBSTANCES

Extracted: dextrose, mannitol
Simultaneous: fructose, galactose

KEY WORDS

SPE; pharmacokinetics

REFERENCE

Marsilio,R.; D'Antiga,L.; Zancan,L.; Dussini,N.; Zaccello,F. Simultaneous HPLC determination with light-scattering detection of lactulose and mannitol in studies of intestinal permeability in pediatrics, *Clin.Chem.*, **1998**, *44*, 1685–1691.

SAMPLE

Matrix: urine

Sample preparation: Dilute urine 2.5-20 fold with water, add a 1 mL aliquot to 1 mL water containing 250 μg/mL arabinose and 25 μg/mL cellobiose, add 0.5 g washed Amberlite IR-120 H:Amberlite IRA400 Cl 40:60, vortex, centrifuge, filter (0.2 μm), inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 40 HPIC-AS6 (Dionex)
Mobile phase: 150 mM NaOH
Flow rate: 1
Injection volume: 50
Detector: E, Dionex pulsed electrochemical detector, gold working electrode, detection potential -0.05 V, oxidation potential +0.6 V, reduction potential -0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6
Internal standard: arabinose (4), cellobiose (9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: mannitol

REFERENCE

Fleming,S.C.; Kapembwa,M.S.; Laker,M.F.; Levin,G.E.; Griffin,G.E. Rapid and simultaneous determination of lactulose and mannitol in urine, by HPLC with pulsed amperometric detection, for use in studies of intestinal permeability, *Clin.Chem.*, **1990**, *36*, 797–799.

SAMPLE

Matrix: urine
Sample preparation: Centrifuge, dilute 10-20 fold with water, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 6.5 Sugar Pak I cation-exchange in calcium form
Mobile phase: Water containing 1 mL/L of a 50 g/L calcium EDTA solution
Column temperature: 85
Flow rate: 0.5
Injection volume: 20
Detector: RI

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Extracted: mannitol

REFERENCE

Willems,D.; Cadranel,S.; Jacobs,W. Measurement of urinary sugars by HPLC in the estimation of intestinal permeability: evaluation in pediatric clinical practice, *Clin.Chem.*, **1993**, *39*, 888–890.

SAMPLE

Matrix: urine
Sample preparation: 2 mL Urine + 500 mg washed and mixed Duolite ion-exchange resin (BDH), vortex for 10 s, centrifuge at 3000 g for 10 min, filter (0.2 μm) the supernatant, inject an aliquot.

HPLC VARIABLES

Guard column: Direct-Connect polymeric guard column (Alltech)
Column: 250 × 4.6 5 μm Kromasil NH2 (Alltech)
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM

Retention time: 15
Limit of detection: 50 μM

OTHER SUBSTANCES

Extracted: mannitol, L-rhamnose, urea

REFERENCE

Miki,K.; Butler,R.; Moore,D.; Davidson,G. Rapid and simultaneous quantification of rhamnose, mannitol, and lactulose in urine by HPLC for estimating intestinal permeability in pediatric practice, *Clin.Chem.*, **1996**, *42*, 71–75.

SAMPLE

Matrix: urine
Sample preparation: 10 μL Urine + 200 μL reagent, heat at 65° for 16 h, cool to room temperature, inject a 5 μL aliquot of the clear supernatant. (Prepare reagent by dissolving 5 mg Fmoc-hydrazine in 1 mL MeCN, add 10 μL buffer. Buffer was 1.44 M formic acid containing 600 mM NaOH. Prepare Fmoc-hydrazine as follows. Dissolve 1 g 9-fluorenylmethyl chloroformate in 100 mL EtOH, add this solution dropwise with stirring to 10 mL hydrazine hydrate (Caution!

Hydrazine hydrate is a carcinogen!), stir for 30 min, filter off the precipitate, wash it twice with 20 mL portions of ice-cold EtOH, dry at room temperature.)

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. Isopropanol:isobutyl alcohol:water 6:6:88 for 13 min, to 80:0:20 (step gradient), maintain at 80:0:20 for 6 min, re-equilibrate at initial conditions.
Column temperature: 50
Injection volume: 5
Detector: F ex 270 em 315

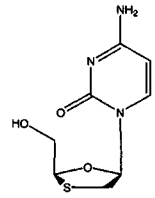
CHROMATOGRAM
Retention time: 5
Limit of detection: 110 nM

OTHER SUBSTANCES
Extracted: 3-O-methyl-D-glucose, rhamnose, xylose

KEY WORDS
derivatization

REFERENCE
Rooyakkers,D.R.; van Eijk,H.M.H.; Deutz,N.E.P. Simple and sensitive multi-sugar-probe gut permeability test by high-performance liquid chromatography with fluorescence labelling, *J.Chromatogr.A*, **1996**, *730*, 99–105.

Lamivudine



Molecular formula: $C_8H_{11}N_3O_3S$
Molecular weight: 229.26
CAS Registry No.: 134678-17-4
Merck Index: 5365
Lednicer No.: 5 99

SAMPLE
Matrix: solutions

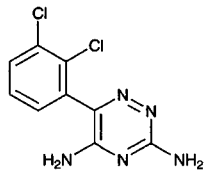
HPLC VARIABLES
Column: 250 × 4.6 Cyclobond I acetyl
Mobile phase: 0.2% Triethylamine in water, adjusted to pH 7.2 with glacial acetic acid
Flow rate: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 6.3 (-), 6.7 (+)

KEY WORDS
chiral

REFERENCE
Coates,J.A.; Cammack,N.; Jenkinson,H.J.; Mutton,I.M.; Pearson,B.A.; Storer,R.; Cameron,J.M.; Penn,C.R. The separated enantiomers of 2'-deoxy-3'-thiacytidine (BCH 189) both inhibit human immunodeficiency virus replication in vitro, *Antimicrob.Agents Chemother.*, **1992**, *36*, 202–205.

Lamotrigine



Molecular formula: $C_9H_7Cl_2N_5$
Molecular weight: 256.09
CAS Registry No.: 84057-84-1 or 84507-84-1 (?)
Merck Index: 5367
Lednicer No.: 4 120

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 30 mg/L IS in water + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 5.5
Internal standard: cyheptamide (14)
Limit of detection: 15-30 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine, carbamazepine diol, carbamazepine epoxide, 5-(p-hydroxyphenyl)-5-phenylhydantoin, phenytoin
Simultaneous: acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS

serum; SPE

REFERENCE

Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

SAMPLE

Matrix: blood
Sample preparation: Mix 550 μL plasma with 100 μL 500 mM monochloroacetic acid in 500 mM pH 2.5 ammonium orthophosphate buffer. Dialyze two 300 μL aliquots against 5 mM pH 7.0 potassium phosphate buffer pumped at 0.25 mL/min for 4 min each using a 15 kD Cuprophan membrane. The dialysate passed through column A and was washed through with 500 μL 1 mM pH 7.0 potassium phosphate buffer. Elute the glucuronide metabolite from column A onto column B with mobile phase. After 30 s remove column A from the circuit, elute column B with mobile phase, elute column A with 200 μL MeCN:water 10:90 and 500 μL 1 mM pH

7.0 potassium phosphate buffer to waste, at the 4 min mark elute lamotrigine and the methylated metabolite from column A onto column B with mobile phase, elute column B with mobile phase, monitor the effluent from column B. (At the end of the process flush the donor channel with 1.5 mL 1 mM pH 7.0 potassium phosphate buffer and flush column A with 500 μL 5 mM pH 7.0 potassium phosphate buffer.)

HPLC VARIABLES

Column: A 70 mg 10 μm Hypersil ODS (Shandon) in a Prelute cartridge; B 150 × 4.6 5 μm Kromasil C8 (Technicol, UK)
Mobile phase: Gradient. A was 50 mM pH 4.15 ammonium phosphate buffer containing 20 mM diethylamine hydrochloride. B was MeCN:500 mM pH 4.15 ammonium phosphate buffer containing 200mM mM diethylamine hydrochloride buffer:water 60:10:30. A:B 86:14 for 3 min, to 69:31 over 0.2 min, maintain at 69:31 for 10.2 min, to 0:100 over 0.1 min, maintain at 0:100 for 1.5 min, return to 86:14 over 0.2 min, re-equilibrate at initial conditions for 3.8 min
Flow rate: 1.5
Detector: UV 270 for 12.5 min, then UV 215

CHROMATOGRAM

Retention time: 9.6
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; column-switching; dialysis

REFERENCE

Cooper,J.D.H.; Shearsby,N.J.; Taylor,J.E.; Fook Sheung,C.T.C. Simultaneous determination of lamotrigine and its glucuronide and methylated metabolites in human plasma by automated sequential trace enrichment of dialysates and gradient high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 227–233.

SAMPLE

Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 μL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 μL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 μm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 μm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4 phosphate buffer 20:80
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM

Retention time: 3.1

OTHER SUBSTANCES

Extracted: oxprenolol

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE
Matrix: blood
Sample preparation: Add an equal volume of 1 μg/mL IS in MeCN to 0.2-1 mL plasma, vortex briefly, add excess sodium carbonate, vortex to form a saturated solution, centrifuge at 6° at 1500 g for 10 min. Remove the MeCN supernatant (top layer) and dilute it by half with water, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100CN
Mobile phase: MeCN:10 mM pH 3.5 ammonium acetate buffer 55:45
Flow rate: 1.5
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 10
Internal standard: 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (A725C) (12)
Limit of detection: 55 ng/mL

OTHER SUBSTANCES
Noninterfering: carbamazepine, clonazepam, phenobarbital, phenytoin, valproic acid

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Alric,R.; Bouvier,O. Performance analysis of a reversed-phase liquid chromatographc assay of lamotrigine in plasma using solvent-demixing extraction, *J.Chromatogr.*, **1991**, *572*, 269–276.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 50 μL 1.7 M perchloric acid, vortex for 20 s, centrifuge at 1500 g for 4 min, add 35 μL 4 M K_2HPO_4, shake gently, allow to settle, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:80 mM KH_2PO_4 25:75
Flow rate: 1.5
Injection volume: 50
Detector: UV 306

CHROMATOGRAM
Retention time: 4.5
Limit of quantitation: 1.2 μM

OTHER SUBSTANCES
Noninterfering: anticonvulsant drugs

KEY WORDS
plasma

REFERENCE
Boutagy,J.; Dell'Anna,M. Simplified and rapid HPLC procedure for analysis of lamotrigine in plasma (Abstract 107), *Ther.Drug Monit.*, **1995**, *17*, 410–410.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 10 μg/mL IS in MeOH + 200 μL 2 M NaOH + 1 mL ethyl acetate, vortex for 1 min, centrifuge for 5 min. Remove the organic layer and evap-

orate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm RP-8 (Brownlee/Applied Biosystems)
Mobile phase: MeCN:water:buffer 20:79:1 (The buffer was 20.7 g $NaH_2PO_4.H_2O$ and 14.2 g Na_2HPO_4 in 500 mL water, pH 6.5.)
Flow rate: 1.6
Injection volume: 25
Detector: UV 306

CHROMATOGRAM
Retention time: 7.2
Internal standard: 6-(2-methoxyphenyl)-1,2,4-triazine-3,5-diamine (BWC430C78) (4)
Limit of detection: 1 μM

OTHER SUBSTANCES
Simultaneous: carbamazepine
Noninterfering: N-acetylprocainamide, ethosuximide, phenobarbital, phenytoin, primidone, procainamide, quinidine, theophylline, valproic acid

KEY WORDS
serum

REFERENCE
Fraser,A.D.; MacNeil,W.; Isner,A.F.; Camfield,P.R. Lamotrigine analysis in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 174–178.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 100 μL MeCN, centrifuge, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 3.5 μm Zorbax SB
Mobile phase: MeCN:MeOH:10 mM pH 7.1 phosphate buffer 7:34:59
Flow rate: 1.5
Injection volume: 5
Detector: UV 310

CHROMATOGRAM
Retention time: 1.9
Limit of detection: <1 μM

OTHER SUBSTANCES
Extracted: carbamazepine (UV 220), carbamazepine epoxide (UV 220), hydroxycarbamazepine (UV 220), oxcarbazepine (UV 220), phenobarbital (UV 220), phenytoin (UV 220)
Also analyzed: ibuprofen, naproxen, trimethoprim

KEY WORDS
plasma

REFERENCE
Lessing,U.; Vielemeyer,O.; Heilmann,P.; Schöneshöfer,M. Routine determination of serum primidone levels with a fully automated liquid chromatographic method: Comparison with an immuno-assay-technique (Abstract 100), *Ther.Drug Monit.*, **1995**, *17*, 408.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut SPE cartridge with two 1 mL portions of MeOH and with two 1 mL portions of buffer. 100 μL Serum + 50 μL 6 μg/mL acetanilide in

MeOH:water 50:50 + 800 μL buffer, mix, add to the SPE cartridge, wash with 1 mL buffer, elute with 250 μL MeOH, inject a 40 μL aliquot. (Buffer was 10 mM pH 3.5 phosphate buffer containing 5 mM sodium octanesulfonate.)

HPLC VARIABLES

Column: 125 × 4 4 μm ODS LiChroCART C18
Mobile phase: MeCN:buffer 27:73 (Buffer was 10 mM pH 3.5 phosphate buffer containing 5 mM sodium octanesulfonate.)
Flow rate: 1
Injection volume: 40
Detector: UV 265

CHROMATOGRAM

Retention time: 10.3
Internal standard: acetanilide (3.4)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, zonisamide
Noninterfering: valproic acid

KEY WORDS

serum; SPE

REFERENCE

Yamashita,S.; Furuno,K.; Kawasaki,H.; Gomita,Y.; Yoshinaga,H.; Yamatogi,Y.; Ohtahara,S. Simple and rapid analysis of lamotrigine, a novel antiepileptic, in human serum by high-performance liquid chromatography using a solid-phase extraction technique, *J.Chromatogr.B*, **1995**, *670*, 354–357.

SAMPLE

Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, weigh out amount corresponding to 10.9 mg lamotrigine, add 100 mL MeOH, sonicate for 5 min, centrifuge an aliquot at 3500 g for 15 min. Remove a 5 mL aliquot of the supernatant and make up to 25 mL with mobile phase. Remove a 4 mL aliquot of this solution and add it to 5 mL 5.5 μg/mL in mobile phase, make up to 25 mL with mobile phase, inject a 20 μL aliquot. Plasma. Condition a 3 mL 200 mg Bond Elut C8 SPE cartridge with 1 volume of MeOH and 1 volume of water. 40 μL Plasma + 200 μL 1.1 μg/mL IS in MeOH + 80 μL MeCN, centrifuge at 3500 g for 15 min. Remove the supernatant and evaporate it so as to remove the organic solvents under a stream of nitrogen at 45°, add the residue to the SPE cartridge, wash with 2 volumes of water, elute with 1 volume of 10 mM HCl in MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. Urine. Condition a 3 mL 200 mg Bond Elut C8 SPE cartridge with 1 volume of MeOH and 1 volume of water. 100 μL Urine + 200 μL 1.1 μg/mL IS in MeOH + 200 μL MeCN, centrifuge at 3500 g for 15 min. Remove the supernatant and evaporate it so as to remove the organic solvents under a stream of nitrogen at 45°, add the residue to the SPE cartridge, wash with 2 volumes of water, elute with 1 volume of 10 mM HCl in MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:buffer 28:72 (Buffer was 56 mL 1 M acetic acid and 50 mL 1 M NaOH made up to 1 L with water, pH 5.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 306

CHROMATOGRAM

Retention time: 6.5
Internal standard: 5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (BW725C78) (3.5)
Limit of detection: 1.2 ng (urine), 1.1 ng (plasma)

Limit of quantitation: 3.0 ng (urine), 2.8 ng (plasma)

KEY WORDS
plasma; tablets; SPE

REFERENCE
Papadoyannis,I.N.; Zotou,A.C.; Samanidou,V.F. Solid-phase extraction study and RP-HPLC analysis of lamotrigine in human biological fluids and in antiepileptic tablet formulations, *J.Liq.Chromatogr.*, **1995**, *18*, 2593–2609.

SAMPLE
Matrix: blood, urine
Sample preparation: Whole blood. Condition a 100 mg C18 SPE cartridge (Burdick and Jackson/Baxter) with 2 mL MeOH, 2 mL water, and 1 mL 50 mM pH 1.2 phosphoric acid buffer. 250 µL Whole blood + 750 µL pH 1.2 phosphoric acid buffer containing 15 mM sodium dodecyl sulfate + 50 µL 30 µg/mL IS in MeOH:water 50:50, vortex for 15 s, centrifuge at 13000 g for 7 min, add the supernatant to the SPE cartridge, wash with 300 µL 50 mM phosphoric acid buffer, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen below 40°, reconstitute the residue in 250 µL mobile phase, filter (0.45 µm), inject a 50 µL aliquot. Urine. Acidify urine with 20% acetic acid, filter (0.45 µm). 1 mL Urine + 1 mL 1 M pH 11.0 NaH_2PO_4 + 50 µL 15 µg/mL IS in MeOH:water 50:50, extract twice with 3 mL ethyl acetate:MTBE 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 µL mobile phase, inject an aliquot. (To analyze glucuronide mix 100 µL urine with 400 µL 50 mM pH 1.2 phosphoric acid buffer containing 15 mM sodium dodecyl sulfate, inject a 10-50 µL aliquot (MeCN:buffer 30:70).)

HPLC VARIABLES
Column: 250 × 4.6 5 µm Spherisorb C8
Mobile phase: MeCN:buffer 33:67 (whole blood) or 40:60 (urine) (Buffer was 50 mM pH 2.2 phosphoric acid containing 10 mM sodium dodecyl sulfate.) (After each injection flush with MeCN:buffer 67:33 for 5 min, re-equilibrate for 5 min.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 50
Detector: UV 277

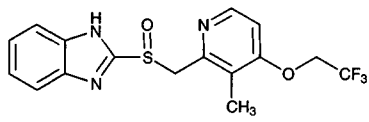
CHROMATOGRAM
Retention time: 19.9 (whole blood), 9.8 (urine)
Internal standard: 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (BW A725C) (11.9 (whole blood), 7.0 (urine))
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: carbamazepine, cyheptamide, desmethyldiazepam, diazepam, ethosuximide, felbamate, lorazepam, oxazepam, phenobarbital, phenytoin, temazepam, tolybarbital

KEY WORDS
guinea pig; whole blood; SPE; pharmacokinetics

REFERENCE
Sinz,M.W.; Remmel,R.P. Analysis of lamotrigine and lamotrigine 2-N-glucuronide in guinea pig blood and urine by reserved-phase ion-pairing liquid chromatography, *J.Chromatogr.*, **1991**, *571*, 217–230.

Lansoprazole



Molecular formula: $C_{16}H_{14}F_3N_3O_2S$
Molecular weight: 369.37
CAS Registry No.: 103577-45-3
Merck Index: 5373
Lednicer No.: 5 115

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 100 μL 25 μg/mL isobutyl p-hydroxybenzoate in dichloromethane + 3 mL diethyl ether:dichloromethane 70:30, extract, centrifuge, repeat extraction. Add 500 μL diethyl ether:dichloromethane: propylene glycol 70:30:0.5 to the supernatants, evaporate to dryness with a stream of nitrogen, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSK gel ODS-120T (Tosoh)
Mobile phase: MeCN:water:n-octylamine 38:62:0.1 adjusted to pH 7 with 85% phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 285

CHROMATOGRAM

Retention time: 11.5
Internal standard: isobutyl p-hydroxybenzoate (23)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; pharmacokinetics

REFERENCE

Aoki,I.; Okumura,M.; Yashiki,T. High-performance liquid chromatographic determination of lansoprazole and its metabolites in human serum and urine, *J.Chromatogr.*, **1991**, *571*, 283–290.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 20 μg/mL n-butyl p-hydroxybenzoate + 100 μL MeOH, vortex for a few s, add 7 mL MTBE, vortex for 45 s, centrifuge at 4° at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, rinse the tube with 1 mL MTBE, evaporate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL MeOH, vortex for 1 min. Dilute a 100 μL aliquot 10-fold with water, inject 1 mL water then the diluted plasma extract onto column A, wash with 1 mL water, elute the contents of column A onto column B with the mobile phase, elute with the mobile phase and monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 mm long 10 μm Nucleosil CN (SFCC); B 10 mm long 5 μm Nucleosil C18 + 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:phosphoric acid:250 mM KH_2PO_4:acetic acid:triethylamine:water 36:10.5:4:0.15:0.15:49.2
Flow rate: 2
Detector: UV 285

CHROMATOGRAM
Retention time: 11
Internal standard: n-butyl p-hydroxybenzoate (23)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acebutolol, allopurinol, amiloride, atenolol, phenobarbital, pindolol, prazosin, quinidine, ranitidine, sotalol

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Landes,B.D.; Miscoria,G.; Flouvat,B. Determination of lansoprazole and its metabolites in plasma by high-performance liquid chromatography using a loop column, *J.Chromatogr.*, **1992**, *577*, 117–122.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 150 μL 4 μg/mL omeprazole in water + 5 mL diethyl ether:dichloromethane 70:30, vortex for 5 min, centrifuge at 6° at 300-850 g for 10 min. Remove 4 mL of the organic layer and evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 500 μL buffer, refrigerate until injection, inject a 100 μL aliquot. (Buffer was MeCN:water 35:65 containing 1 mL/L n-octylamine and 5 mM N-acetohydroxamic acid, pH adjusted to 7.5 with 85% phosphoric acid.)

HPLC VARIABLES
Column: 150 or 250 × 4.6 5 μm Hi-Chrom Reversible octadecylsilane (Regis)
Mobile phase: MeCN:water 35:65 containing 1 mL/L n-octylamine and 5 mM N-acetohydroxamic acid, pH adjusted to 7.0 with 85% phosphoric acid
Column temperature: 40-43
Flow rate: 1 for 15 min then 2.5
Injection volume: 100
Detector: UV 285

CHROMATOGRAM
Retention time: 13.2
Internal standard: omeprazole (7.6)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Karol,M.D.; Granneman,G.R.; Alexander,K. Determination of lansoprazole and five metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *668*, 182–186.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.613

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in EtOH, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralpak AD (Daicel)
Mobile phase: Hexane:EtOH 65:35
Column temperature: 35
Flow rate: 1
Injection volume: 10-20
Detector: UV 302

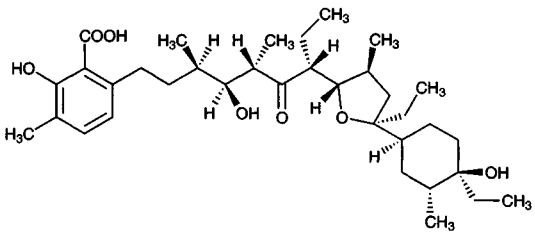
CHROMATOGRAM
Retention time: k' 2.91, α 1.15

OTHER SUBSTANCES
Also analyzed: timoprazole, omeprazole, pantoprazole

KEY WORDS
chiral

REFERENCE
Balmér,K.; Persson,B.-A.; Lagerström,P.-O. Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases, *J.Chromatogr.A*, **1994**, *660*, 269–273.

Lasalocid A



Molecular formula: $C_{34}H_{54}O_8$
Molecular weight: 590.80
CAS Registry No.: 25999-31-9
Merck Index: 5384

SAMPLE

Matrix: blood

Sample preparation: Cow, dog. 10 mL Whole blood + 1 mL 1 M NaOH, shake vigorously for 10-15 s, let stand at room temperature for 5 (cow) or 20 (dog) min, add 20 mL ethyl acetate, shake vigorously by hand for 50-60 s, centrifuge at 350 g for 10-15 min. Remove 1-10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, vortex vigorously for 1 min, inject an aliquot. Chicken. 10 mL Whole blood + 20 mL ethyl acetate, shake vigorously by hand for 10-15 s, shake on a reciprocal shaker at high speed for 5 min, centrifuge at 350 g for 10-15 min. Remove 1-10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, vortex vigorously for 1 min, inject an aliquot. Rat, mouse. 1 mL Whole blood + 10 mL ethyl acetate, shake vigorously by hand for 30-40 s, shake on a reciprocal shaker at high speed for 5 min, shake vigorously by hand for 10-15 s, centrifuge at 350 g for 10-15 min. Remove 9 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, vortex vigorously for 1 min, inject an aliquot.

HPLC VARIABLES

Column: 250 mm long 5 μm Partisil PXS 5/25

Mobile phase: Hexane:THF:MeOH:triethylamine:ammonium hydroxide 81:14:2:2:1 shake for 30-40 s, let stand for 25-30 min, discard the lower phase, add 10 mL/L THF, do not degas or filter. (Place a 10 μm Partisil column before the injector. Flush system with hexane for 30 min at 2 mL/min at the end of each day.)

Flow rate: 0.9

Injection volume: 42-100

Detector: F ex 310 em 430

CHROMATOGRAM

Retention time: 6-8

Limit of detection: <5 ppb

KEY WORDS

whole blood; mouse; rat; cow; dog; chicken; normal phase

REFERENCE

Kaykaty,M.; Weiss,G. Lasalocid determination in animal blood by high-performance liquid chromatography fluorescence detection, *J.Agric.Food Chem.*, **1983**, *31*, 81–84.

SAMPLE

Matrix: feed, premix

Sample preparation: Premix. 2 g Premix + 100 mL acidified MeOH, sonicate at 40° for 2 min, cool, make up to 250 mL with acidified MeOH, mix thoroughly, let stand for 1 h, filter (0.45 μm) an aliquot, dilute the filtrate with acidified MeOH to produce a 4 μg/mL solution, inject a 20 μL aliquot. Feed. Grind feed to pass a 1 mm sieve, add 100 mL acidified MeOH, sonicate at 40° for 2 min, cool, make up to 250 mL with acidified MeOH, mix thoroughly, let stand for 1 h, filter (0.45 μm) an aliquot, inject a 20 μL aliquot of the filtrate. (Acidified MeOH was MeOH:concentrated HCl 99.5:0.5.)

HPLC VARIABLES

Column: 250 or 125 × 4 5 μm C18

Mobile phase: MeOH:buffer 95:5 (Prepare buffer by dissolving 1.36 g KH_2PO_4 in 500 mL water, add 3 mL phosphoric acid, add 10 mL 1,5-dimethylhexylamine, adjust pH to 4.0 with 20% phosphoric acid, make up to 1 L with water.)
Flow rate: 1.2
Injection volume: 20
Detector: F ex 310 em 419

CHROMATOGRAM
Retention time: 7

REFERENCE
Analytical Methods Committee, Determination of lasalocid sodium in poultry feeds and premixes, *Analyst*, **1995**, *120*, 2175–2180.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Mega Bond Elut silica gel SPE cartridge with benzene (Caution! Benzene is a carcinogen!). Evaporate a solution in MeOH to dryness, add 5 mL 5.28 mg/mL 1-bromoacetylpyrene in MeCN, add 5 mL 1.28 mg/mL Kryptofix 222 in MeCN, heat at 50° for 1.5 h, cool. Either inject this solution directly or evaporate it to dryness, dissolve the residue in 5 mL benzene:chloroform 50:50, rinse out the flask with two 5 mL portions of benzene:chloroform 50:50, filter, add the filtrate to the SPE cartridge, elute with two 5 mL portions of benzene:acetone 70:30. Evaporate the eluate to dryness, reconstitute the residue in 10 mL MeCN, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Develosil 5C18
Mobile phase: MeOH:water 97:3
Flow rate: 1
Detector: F ex 360 em 420

CHROMATOGRAM
Retention time: 15
Internal standard: 18,19-dihydrosalinomycin (25), 18,19-dihydro-20-ketosalinomycin (16.5)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: narasin, monensin, salinomycin

KEY WORDS
derivatization; SPE

REFERENCE
Asukabe,H.; Murata,H.; Harada,K.-I.; Suzuki,M.; Oka,H.; Ikai,Y. Improvement of chemical analysis of antibiotics. XX. Basic study on high-performance liquid chromatographic determination of four polyether antibiotics pre-derivatized with 1-bromoacetylpyrene, *J.Chromatogr.A*, **1993**, *657*, 349–356.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Tissuemizer) 10 g tissue and 25 mL solvent for 1 min, wash blades with 3-4 mL solvent, combine with homogenate, shake for 30 min, centrifuge at 800 g for 15 min, decant. Add 25 mL solvent to residue, mix thoroughly, shake vigorously for 1 min, centrifuge at 800 g for 15 min, add supernatants to a 75 × 20 column of 80-200 mesh alumina (Fisher), rinse container onto column with 25 mL solvent, add 100 mL solvent to the column. Combine all the eluates and add 100 mL 5% NaCl, shake vigorously, let stand 2-3 min, add 30 mL dichloromethane, shake vigorously for 30 s, repeat extraction twice. Combine the dichloromethane layers and evaporate them to dryness under reduced pressure at 48-50°, reconstitute the residue in 1 mL solvent, add to a 75 × 20 column of 25-100 μm Sephadex LH-20, rinse flask with two 3.5 mL portions of solvent and add the rinses to the column, add 10 mL solvent to the column, discard the first 18 mL of eluate, add 10 mL solvent to the column, collect this fraction, evaporate to dryness under a stream of nitrogen at 48-50°, make up to 1 mL with solvent, mix, add 500 μL 9-anthryldiazomethane solution, let stand in the dark for

30 min, evaporate to dryness under a stream of nitrogen at 48-50°, reconstitute in 1 mL hexane. Condition a Baker-10 silica SPE cartridge with 5-10 mL hexane, do not allow to dry. Add the hexane solution to the SPE cartridge, rinse tube with 9 mL hexane, add rinse to the SPE cartridge. Wash SPE cartridge with 10 mL hexane:dichloromethane 50:50, with 10 mL hexane: dichloromethane 20:80, with 10 mL dichloromethane, and with 1 mL MeOH. Elute with 1 mL MeOH, inject a 20 μL aliquot of the eluate. (Solvent was MeOH:water 80:20. Prepare 9-anthryldiazomethane solution as follows. Add 1100 g manganous sulfate tetrahydrate in 1.5 L water and 1170 mL 40% NaOH over 1 h to a hot stirred solution of 960 g potassium permanganate in 6 L water, stir for 1 h, centrifuge, wash solid with water until washings are colorless, dry solid at 100-120°, grind the activated manganese dioxide to a fine powder. Add 8.5 g 85% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) to 8.8 g 9-anthraldehyde dissolved in 150 mL EtOH, stir at room temperature for 3 h, filter off solid, dry under vacuum, recrystallize from EtOH to give 9-anthraldehyde hydrazone as red-yellow crystals, mp 124-6°. Dissolve 220 mg 9-anthraldehyde hydrazone in 100 mL anhydrous ethyl ether, add 800 mg activated manganese dioxide, add 600 μL EtOH saturated with KOH, stir vigorously for 30 min, filter (glass fiber), wash solid with 20 mL anhydrous ethyl ether, evaporate to reduce volume, make up to 100 mL with anhydrous ethyl ether, store in a dark flask in the dark in a refrigerator. Discard after 30 days (J.Assoc.Off.Anal.Chem. 1985, 68, 1149).)

HPLC VARIABLES
Guard column: pellicular C18 (Alltech)
Column: 200 × 4.6 5 μm RP-C8 (Hewlett-Packard)
Mobile phase: Gradient. A was MeCN. B was MeCN:water 10:90. A:B 20:80 for 9 min, 10:90 for 7 min, 20:80 for 1 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 418 (filter)

CHROMATOGRAM
Retention time: 12.6
Limit of detection: 0.15 ppm

OTHER SUBSTANCES
Extracted: monensin, salinomycin

KEY WORDS
cow; liver; SPE; protect from light; derivatization

REFERENCE
Martinez,E.E.; Shimoda,W. Liquid chromatographic determination of multiresidue fluorescent derivatives of ionophore compounds, monensin, salinomycin, narasin, and lasalocid, in beef liver tissue, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 637–641.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g minced liver and 40 mL MeCN for 15-30 s, scrape down sides and shaft, homogenize for 15-30 s, centrifuge at 5-10° at 2000-2500 rpm for 15-20 min, decant 23 mL supernatant, add 23 mL hexane, shake vigorously for 15-20 s, centrifuge at 1500-2000 rpm. Evaporate 20 mL of the lower MeCN layer to dryness under a stream of nitrogen at 55-65°, add 1 mL water saturated with mobile phase, vortex for 15-20 s, add 2 mL mobile phase, vortex for 15-20 s, centrifuge at 2000-2500 rpm for 15-20 min, recentrifuge if top layer is not clear, inject an aliquot of the top layer.

HPLC VARIABLES
Column: Two Partisil 10 PXS 10/25 columns in series
Mobile phase: THF:MeOH:hexane:mixture 3.75:0.75:20.5:75, do not filter or degas. (Mixture was 150 mL THF + 30 mL MeOH + 10 mL ammonium hydroxide + 810 mL hexane, mix, let stand for 1 h, discard lower layer.) (Place a silica column between pump and injector.)
Flow rate: 2
Injection volume: 50
Detector: F ex 310 em 430

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.24-0.47 ppm

KEY WORDS
normal phase; liver; cow

REFERENCE
Newkirk,D.R.; Barnes,C.J. Liquid chromatographic determination and gas chromatographic-mass spectrometric confirmation of lasalocid sodium in bovine liver: interlaboratory study, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 581–584.

SAMPLE
Matrix: tissue
Sample preparation: 10 g Minced tissue + 50 mL MeCN, homogenize for 2 min, sonicate for 5 min, centrifuge at 1860 g for 5 min, repeat extraction. Combine the supernatants and add 30 mL carbon tetrachloride and 20 mL saturated NaCl, shake for 1 min, filter the organic layer through anhydrous sodium sulfate and phase separating paper, evaporate to dryness. Transfer the residue to a Bond-Elut silica SPE cartridge with three 2 mL aliquots of hexane, wash with 5 mL chloroform, elute with 10 mL chloroform:MeOH 95:5. Evaporate the eluate to dryness, transfer to a small vial with three 2 mL portions of hexane, evaporate to dryness with a stream of nitrogen, reconstitute in 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S styrene-divinylbenzene copolymer (Polymer Labs)
Column: 250 × 4.6 5 μm PLRP-S styrene-divinylbenzene copolymer (Polymer Labs)
Mobile phase: MeCN:10 mM pH 10.0 disodium tetraborate (borax) 60:40
Flow rate: 1
Injection volume: 10
Detector: F ex 310 em 430

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 2 ng/g

KEY WORDS
chicken; muscle; SPE

REFERENCE
Tarbin,J.A.; Shearer,G. Improved high-performance liquid chromatographic procedure for the determination of lasalocid in chicken tissues and egg using polymeric and porous graphitic carbon columns, *J.Chromatogr.*, **1992**, *579*, 177–183.

SAMPLE
Matrix: tissue, eggs
Sample preparation: 2 g Minced tissue or egg + 25 mL MeCN, homogenize for 2 min, sonicate for 5 min, centrifuge at 1860 g for 5 min, repeat extraction. Combine the supernatants and add 50 mL carbon tetrachloride and 20 mL saturated NaCl, shake for 1 min, filter the organic layer through anhydrous sodium sulfate and phase separating paper, evaporate to dryness. Transfer the residue to a Bond-Elut silica SPE cartridge with three 2 mL aliquots of hexane, wash with 5 mL chloroform, elute with 10 mL chloroform:MeOH 95:5. Evaporate the eluate to dryness, transfer to a small vial with three 2 mL portions of hexane, evaporate to dryness with a stream of nitrogen, reconstitute in 500 μL mobile phase, inject a 25 μL aliquot.

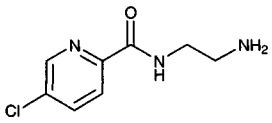
HPLC VARIABLES
Column: 100 × 4.6 7 μm Hypercarb porous graphitic carbon (Shandon)
Mobile phase: MeCN containing 5% 1,1,3,3-tetramethylguanidine
Flow rate: 0.5
Injection volume: 25
Detector: F ex 310 em 420

CHROMATOGRAM
Retention time: 7
Limit of detection: 10 ng/g (eggs), 2 ng/g (tissue)

KEY WORDS
chicken; muscle; SPE

REFERENCE
Tarbin,J.A.; Shearer,G. Improved high-performance liquid chromatographic procedure for the determination of lasalocid in chicken tissues and egg using polymeric and porous graphitic carbon columns, *J.Chromatogr.*, **1992**, *579*, 177–183.

Lazabemide



Molecular formula: $C_8H_{10}ClN_3O$
Molecular weight: 199.64
CAS Registry No.: 103878-84-8, 103878-83-7 (HCl)
Merck Index: 5407

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 400 mM NaOH, vortex, add 10 mL MTBE:1-butanol 80:20, shake at 30 rpm on a rotating shaker for 20 min, centrifuge at 2000 g for 5 min. Remove 9 mL of the organic layer and add it to 500 μL 0.17% phosphoric acid, extract for 20 min, centrifuge for 5 min. Remove the aqueous layer and add it to 500 μL buffer, add 500 μL 50 μg/mL fluorescamine in MeCN (prepare fresh each day) with constant vortexing, after 10 min remove the MeCN by evaporation under reduced pressure for exactly 10 min, vortex, inject a 100 μL aliquot. (Prepare buffer by dissolving 30 g Na_2HPO_4 in water, add 24 mL 1 M NaOH, make up to 1 L with water.)

HPLC VARIABLES
Column: 125 × 4 LiChroCART Superspher 60 RP-8e (Merck)
Mobile phase: MeCN:water 32:68 containing 100 mM NaH_2PO_4 and 5 mM Na_2HPO_4, pH 5.9 ± 0.1
Flow rate: 1
Injection volume: 100
Detector: F ex 370 em 485

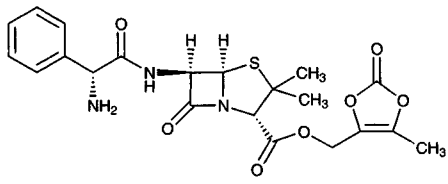
CHROMATOGRAM
Retention time: 3.7
Limit of detection: 0.5 ng/mL
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Noninterfering: benserazide, dopamine, levodopa

KEY WORDS
derivatization; plasma; rat; human

REFERENCE
Wyss,R.; Philipp,W. Determination of the monoamine oxidase B inhibitor Ro 19-6327 in plasma by high-performance liquid chromatography using precolumn derivatization with fluorescamine and fluorescence detection, *J.Chromatogr.*, **1990**, *507*, 187–198.

Lenampicillin



Molecular formula: $C_{21}H_{23}N_3O_7S$
Molecular weight: 461.50
CAS Registry No.: 86273-18-9
Merck Index: 5460

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 1 mL MeOH, stir for 5 min, centrifuge at 2400 g for 10 min. Remove 1 mL supernatant, add 2 μg cefazolin, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:67 mM KH_2PO_4 20:80
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 9 (measured as ampicillin peak)
Internal standard: cefazolin (14)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Marzo,A.; Monti,N.; Ripamonti,M.; Arrigoni Martelli,E.; Picari,M. High-performance liquid chromatographic assay of ampicillin and its prodrug lenampicillin, *J.Chromatogr.*, **1990**, *507*, 235–239.

SAMPLE
Matrix: solutions

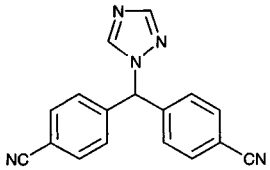
HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:67 mM KH_2PO_4 35:65
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 20
Internal standard: o-tolylpiperazine (8.5)
Limit of detection: 1500 ng/mL

OTHER SUBSTANCES
Simultaneous: ampicillin

REFERENCE
Marzo,A.; Monti,N.; Ripamonti,M.; Arrigoni Martelli,E.; Picari,M. High-performance liquid chromatographic assay of ampicillin and its prodrug lenampicillin, *J.Chromatogr.*, **1990**, *507*, 235–239.

Letrozole



Molecular formula: $C_{17}H_{11}N_5$
Molecular weight: 285.31
CAS Registry No.: 112809-51-5
Merck Index: 5474

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with 50 μL 6.12 μM IS, adjust to pH 13.0, add to an Extrelut SPE cartridge. Elute with 6 mL and 5 mL portions of dichloromethane:diethyl ether 40:60, evaporate the eluates to dryness, dissolve the residue in 500 μL pH 7.0 phosphate buffer, wash with 2 mL hexane, inject a 120 μL aliquot of the aqueous phase.

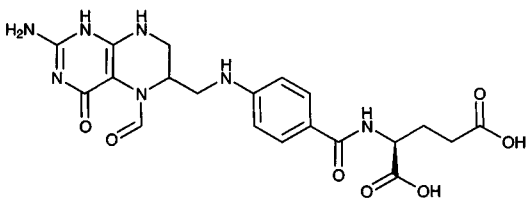
HPLC VARIABLES
Column: 5 μm LiChrospher RP 8
Mobile phase: MeCN:pH 7.0 phosphate buffer 42:58
Flow rate: 1
Injection volume: 120
Detector: UV 234

CHROMATOGRAM
Internal standard: CGS 18320 B (4,4'-[1H-1,3-diazol-1-ylmethylene]bis-benzonitrile)
Limit of quantitation: 8.9 nM

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Sioufi,A.; Sandrenan,N.; Godbillon,J.; Trunet,P.; Czendlik,C.; Howald,H.; Pfister,C.; Ezzet,F. Comparative bioavailability of letrozole under fed and fasting conditions in 1 healthy subjects after a 2.5 mg single oral administration, *Biopharm.Drug Dispos.*, **1997**, *18*, 489–497.

Leucovorin



Molecular formula: $C_{20}H_{23}N_7O_7$
Molecular weight: 473.45
CAS Registry No.: 58-05-9, 1492-18-8 (Ca salt), 6035-45-6 (Ca salt pentahydrate)
Merck Index: 4254

SAMPLE
Matrix: blood
Sample preparation: Mix 20 μL serum with 20 μL 2 M perchloric acid, vortex for a few seconds, centrifuge at 10000 g for 2 min. Inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (GL Sciences, Japan)
Mobile phase: MeCN:25 mM pH 7.0 sodium phosphate buffer 8:92 containing 25 mM hydrogen peroxide
Flow rate: 1
Injection volume: 20
Detector: F ex 379 em 457 following post-column reaction. The column effluent flowed through a 5 m × 0.5 mm i.d. stainless-steel reaction coil at 160° and a 3 m × 0.5 mm i.d. stainless-steel coil at 15° to the detector.

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: methotrexate

KEY WORDS
serum; post-column reaction

REFERENCE
Kubo,H.; Umiguchi,Y.; Fukumoto,M.; Kinoshita,T. Fluorometric determination of methotrexate in serum by high-performance liquid chromatography using in-line oxidation with hydrogen peroxide, *Anal.Sci.*, **1992**, *8*, 789–792.

SAMPLE
Matrix: blood
Sample preparation: Add 2 mg ascorbic acid to each 1 mL blood, centrifuge at 800 g in the cold. 1 mL Plasma + 10 μL 30 μg/mL methotrexate in 1 mg/mL ascorbic acid + 1.5 mL MeOH, vortex, centrifuge at 800 g. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, inject the whole amount.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. A was 250 mM pH 5.0 phosphate buffer. B was MeOH:250 mM pH 5.0 phosphate buffer 1:1. A:B 100:0 for 15 min, to 50:50 over 15 min, to 0:100 over 5 min, maintain at 0:100 for 5 min.
Flow rate: 2
Injection volume: 200
Detector: UV 310

CHROMATOGRAM
Retention time: 21
Internal standard: methotrexate (29)

KEY WORDS
plasma

REFERENCE
Wainer,I.W.; Stiffin,R.M. Direct resolution of the stereoisomers of leucovorin and 5-methyltetrahydrofolate using a bovine serum albumin high-performance liquid chromatographic chiral stationary phase coupled to an achiral phenyl column, *J.Chromatogr.*, **1988**, *424*, 158–162.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut RP-18 SPE cartridge with four 2 mL portions of MeOH and three 2 mL portions of 100 mM pH 4.7 TrisP buffer. Add 1 mg/mL ascorbic acid to plasma. Dilute 500 μL plasma six fold with 9 mg/mL NaCl containing 1 mg/mL ascorbic acid, add 200 μL 8.5 mg/mL phosphoric acid, add to the SPE cartridge, wash with 500 μL 10 mM pH 4.7 TrisP buffer, elute with 1.5 mL eluant. Evaporate the eluate to dryness under a stream of nitrogen at 37° for 30 min, reconstitute the residue in 250 μL 9 mg/mL NaCl containing 1 mg/mL ascorbic acid, inject a 40 μL aliquot onto column A and elute with mobile phase, collect 600 μL effluent (monitor with detector A) in a sample loop and inject it onto column B, elute with mobile phase and monitor the effluent from column B with detector B. (TrisP was tris(hydroxyethyl)methylaminomethane phosphate. Eluant was MeOH:10 mM TrisP buffer 75:25, pH 7 containing 150 μg/mL ascorbic acid.)

HPLC VARIABLES
Column: A 119 × 2 4 μm Superspher RP-8 (Merck) (Condition column with MeOH:water 30:70 every 100-200 injections.); B 150 × 4.5 7 μm chiral protein 2 HSA-BP human serum albumin (Société Française Chromato Colonne)

Mobile phase: 1-Propanol:200 mM Na_2HPO_4 2:98 adjusted to pH 6.2 with 8.5 g/L phosphoric acid
Column temperature: 35 (column B)
Flow rate: A 0.25; B 1
Injection volume: 40
Detector: A UV 313; B E, ESA Coulochem 5100 A, Model 5020 guard cell, Model 5010 analytical cell, E1 0.30 V, E2 0.55 V (monitored), guard cell is placed before second injection valve

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CHROMATOGRAM
Retention time: 6 (l), 10 (d) (after injection onto column B)

KEY WORDS
plasma; narrow bore; column-switching; heart cut; chiral; SPE

REFERENCE
Etienne,M.-C.; Speziale,N.; Milano,G. HPLC of folinic acid diastereoisomers and 5-methyltetrahydrofolate in plasma, *Clin.Chem.*, **1993**, *39*, 82–86.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bakerbond C18 SPE cartridge with 1 mL MeOH and 1 mL 1% acetic acid. 1 mL Plasma + 1 mL 5% acetic acid, add to the SPE cartridge, elute with MeCN:pH 7.0 phosphate buffer (ionic strength 0.1) 20:80, inject a 100 µL aliquot of the eluate onto column A with mobile phase A. Collect the fraction containing leucovorin (about 8 min) in a 250 µL sample loop and inject onto column B with mobile phase B, monitor the effluent from column B. (For achiral determination use only column A and mobile phase A, LOQ 50 ng/mL.)

HPLC VARIABLES
Column: A 5 µm Novapack C18 pre-column + 300 × 4 5 µm Novapack RP-18; B 7 µm Resolvosil BSA chiral pre-column + 150 × 4 7 µm Resolvosil BSA chiral (Macherey-Nagel)
Mobile phase: A Gradient. MeCN:pH 7.0 phosphate buffer (ionic strength 0.1) from 0:100 to 30:70 over 10 min, to 100:0 over 8 min.; B pH 7.0 phosphate buffer (ionic strength 0.1)
Flow rate: A 1; B 0.5
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 19 (S), 22 (R)

KEY WORDS
plasma; SPE; chiral; column-switching; heart-cut

REFERENCE
Vandenbosch,C.; van Belle,S.; de Smet,M.; Taton,G.; Bruynseels,V.; Vandenhoven,G.; Massart,D.L. Determination of leucovorin and 5-fluorouracil in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 77–85.

SAMPLE
Matrix: blood, CSF
Sample preparation: 250 µL Plasma or CSF + 25 µL 10 mg/mL ascorbic acid + 250 µL ice-cold 1.5 M perchloric acid, vortex, let stand in ice-water for 5 min, centrifuge at 4° at 3000 g for 5 min. Remove 350 µL of the supernatant with 50 µL 8 M potassium acetate, keep in ice-water for 2 min, centrifuge at 4° at 3000 g for 2 min, inject a 100 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 200 × 3 5 µm Hypersil ODS glass column
Mobile phase: Gradient. A was 10 mM ammonium formate adjusted to pH 3.5 with HCl. B was MeCN:10 mM ammonium formate 25:75 adjusted to pH 3.5 with HCl. A:B from 85:15 to 5:95 over 21 min, maintain at 5:95 for 1 min, re-equilibrate at initial conditions for 11 min.
Flow rate: 0.4

Injection volume: 100
Detector: UV 305

CHROMATOGRAM
Retention time: 15
Limit of detection: 200 nM

OTHER SUBSTANCES
Extracted: methotrexate, N^5-methyltetrahydrofolate

KEY WORDS
plasma

REFERENCE
van Tellingen,O.; van der Woude,H.R.; Beijnen,J.H.; van Beers,C.J.T.; Nooyen,W.J. Stable and sensitive method for the simultaneous determination of N^5-methyltetrahydrofolate, leucovorin, methotrexate and 7-hydroxymethotrexate in biological fluids, *J.Chromatogr.*, **1989**, *488*, 379–388.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 500 μL Serum + 250 μg ascorbic acid + 750 μL MeCN, vortex, centrifuge at 400 g for 1 min, add 7 mL chloroform, mix, centrifuge at 400 g for 1 min. 400 μL Aqueous phase + 2 mL mobile phase A, inject the whole amount through a 125 × 4 40 μm silica (Supelco) column on to column A and elute to waste with mobile phase A, backflush the contents of column A on to column B and elute to waste with mobile phase B, collect the fraction containing leucovorin in a 1 mL sample loop, inject this fraction on to column C and elute with mobile phase C, monitor the effluent from column C. Urine. Condition a 1 mL C18 SPE cartridge (Supelco) with 2 mL water, 2 mL MeOH, and 2 mL mobile phase A. Dilute urine with 4 volumes of mobile phase A, add to the SPE cartridge, wash with 2 mL mobile phase A, elute with 2 mL MeCN:water 50:50. Wash the eluate with chloroform, filter (Waters ultrafiltration cartridge) the aqueous layer while centrifuging, dilute 400 μL of the ultrafiltrate with 2 mL mobile phase A, inject the whole amount through a 125 × 4 40 μm silica (Supelco) column on to column A and elute to waste with mobile phase A, backflush the contents of column A on to column B and elute to waste with mobile phase B, collect the fraction containing leucovorin in a 1 mL sample loop, inject this fraction on to column C and elute with mobile phase C, monitor the effluent from column C.

HPLC VARIABLES
Column: A 30 × 4 5 μm C18; B 250 × 2 3 μm C18 (Macherey-Nagel); C 150 × 4.6 7 μm Resolvosil BSA-7
Mobile phase: A 5 mM tetrabutylammonium phosphate (low-UV Pic A) adjusted to pH 6.5 with phosphoric acid; B Isopropanol:buffer 7.5:92.5 adjusted to pH 5 with phosphoric acid (Buffer was 1.5 mM sodium phosphate containing 0.75 mM tetrabutylammonium phosphate (low-UV PIC A).); C 28 mM Phosphate buffer containing 0.6 mM sodium azide (Caution! Sodium azide is toxic! Do not discharge to plumbing system!)
Column temperature: 40 (B and C only, A is ambient)
Flow rate: A 2; B 0.15; C 0.4
Injection volume: 2400
Detector: F ex 308 em 365

CHROMATOGRAM
Retention time: 26 (6S), 30 (6R)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 5-methyltetrahydrofolate

KEY WORDS
serum; chiral; column-switching; heart-cut; SPE; pharmacokinetics

REFERENCE
Schleyer,E.; Reinhardt,J.; Unterhalt,M.; Hiddemann,W. Highly sensitive coupled-column high-performance liquid chromatographic method for the separation and quantitation of the diastereomers of leucovorin and 5-methyltetrahydrofolate in serum and urine, *J.Chromatogr.B*, **1995**, *669*, 319–330.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeOH:buffer 21:79 (Buffer was 15 mL 1 M tetrabutylammonium hydroxide in MeOH + 850 mL water, pH adjusted to 7.5 ± 0.1 with 2 M NaH_2PO_4, make up to 875 mL with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4

KEY WORDS
stability-indicating; saline; injections

REFERENCE
Smith,J.A.; Morris,A.; Duafala,M.E.; Bertino,J.R.; Markman,M.; Kleinberg,M. Stability of floxuridine and leucovorin calcium admixtures for intraperitoneal administration, *Am.J.Hosp.Pharm.*, **1989**, *46*, 985–989.

SAMPLE
Matrix: solutions
Sample preparation: Prepare aqueous solutions, stabilize them with ascorbic acid (1 mg/mL water), flush with nitrogen, refrigerate, keep in brown glass vials. Inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Lichrospher 60 RP-select B
Column: 125 × 3 3 μm Hypersil BDS
Mobile phase: Gradient. A was MeCN. B was 5 mM monobasic potassium phosphate adjusted to pH 2.3 with phosphoric acid. A:B 7:93 for 5 min, to 13:87 over 15 min, to 21:79 over 6 min, maintain at 21:79 for 1 min, back to 7:93 over 2 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 0.5
Injection volume: 10
Detector: F ex 295 em 355 following post-column photochemical derivatization. The column effluent flowed through a 10 m × 0.3 mm ID Teflon tube irradiated with a 254 nm mercury lamp to the detector.

CHROMATOGRAM
Retention time: 14
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, methotrexate

KEY WORDS
post-column photochemical derivatization; post-column reaction

REFERENCE
Mandl,A.; Lindner,W. Improved detection of leucovorin in mixed folates and antifolates by reversed-phase liquid chromatography and on-line post-column UV irradiation, *Chromatographia*, **1996**, *43*, 327–330.